Exploring the Link between Diet and Cancer

THE link between the human diet and cancer presents an intriguing puzzle for scientists. Research indicates that different foods and how they are prepared can increase a person's risk for cancer. Cooked muscle meats such as beef, pork, and fowl are one such food. They contain a class of tumor-causing mutagens called heterocyclic amines that target specific organs. For example, 2-amino-1-methyl-6-phenylimidazol[4,5-*b*]pyridine (PhIP), the most abundant mutagen produced in cooked foods, may cause tumors in the breast, prostate, and colon of laboratory animals. Scientists have yet to explain the connection, but a Livermore team funded by the National Cancer Institute is finding new evidence to solve this puzzle.

Led by biomedical scientist Kristen Kulp, the team is combining breast cell–based assays with computational modeling and nuclear magnetic resonance (NMR) to better understand the cell-growth process. This work is part of the Laboratory's ongoing research on diet and cancer, which is funded by the National Institutes of Health and the Department of Defense's Breast Cancer Research Program. Results from the team's experiments and simulations indicate that PhIP competes with the estrogen hormone estradiol, thereby disrupting the hormone's regulatory role in cell growth.

Livermore's studies of food mutagens are a spin-off from early efforts to understand the human health hazards of coal gasification and oil-shale retorting. Curiously, the chemical processes that occur when oil shale is heated are similar to those occurring when food is cooked, and both processes produce mutagenic arylamines. In the 1970s, the National Institute of Environmental Health, learning of the oil-shale project, asked the Laboratory to use its expertise to study food mutagens.

An Attempt to Displace Estrogen

Heterocyclic amines form when amino acids, creatine (a chemical found in muscles), and sugar react at high cooking temperatures. Researchers have identified 17 heterocyclic amines that may pose a cancer risk in humans. Kulp's team is studying three of them—PhIP, 2-amino-3,8-dimethylimidazo[4,5-*f*] quinoxaline (MeIQx), and 2-amino-1,6-dimethylfuro[3,2-*e*] imidazo[4,5-*b*]pyridine (IFP)—as well as a PhIP metabolite called N^2-hydroxy-PhIP.

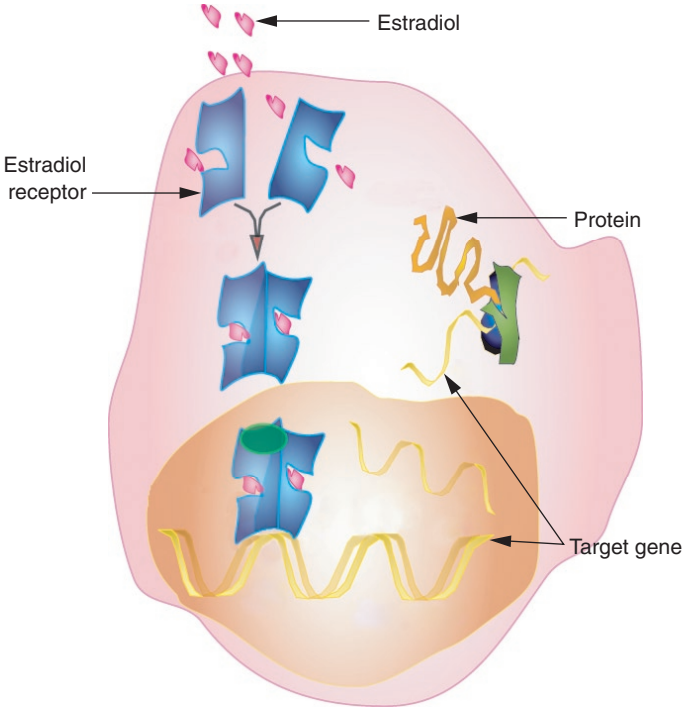
PhIP is similar in structure to the hormone estradiol, which stimulates cell growth in the breast and other parts of the body by binding to estrogen-receptor proteins. Estrogen receptors also modulate gene expression in the developing fetus and in adolescents as secondary sexual characteristics develop. Estrogen receptors belong to a class of proteins called nuclear receptors, all of which contain a ligand-binding domain. When estradiol binds to this domain in an estrogen receptor, it activates the receptor.

Two phases in the metabolic pathway of PhIP mimic that of estradiol. During the first phase, PhIP oxidizes into a hydroxylated intermediate, N^2-hydroxy-PhIP. In the second phase, metabolizing enzymes convert N^2-hydroxy-PhIP to a more biologically reactive form, generating esters that can bind DNA and cellular proteins. "The body converts heterocyclic amines into a water-soluble form that can be excreted," says Kulp. "The reactive compounds formed in this process can attach to DNA and cause mutations that are believed to lead to cancer. In addition to the cell damage caused through this pathway, we've also discovered that PhIP binds directly to the estrogen receptor and may play a role in regulating cell growth."

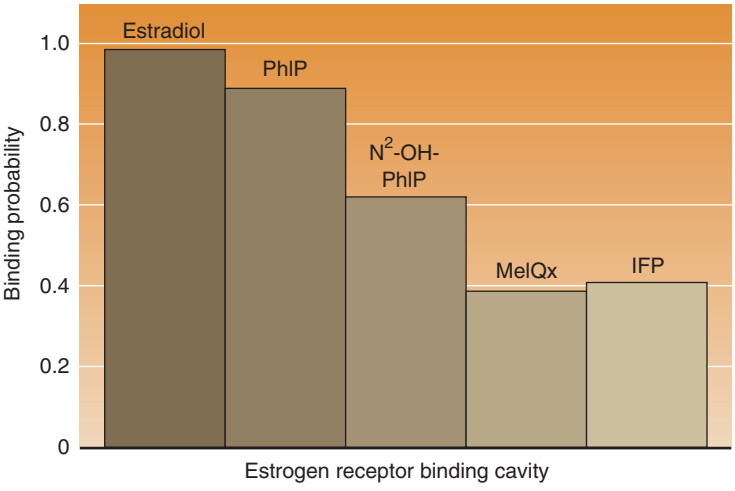
The team's results could explain the tumor specificity in certain organs. Direct binding to the estrogen receptor's ligand-binding domain may promote cancer. "When PhIP activates the estrogen receptor and related cell-growth pathways, it may enhance the damage by accelerating the rate at which mutated cells duplicate," says Kulp.

Simulating the Positions of Molecules

To study potential binding interactions between heterocyclic amines and the estrogen receptor, Kulp's team generated models



The hormone estradiol stimulates cell growth in the breast and other parts of the body by binding to an estrogen-receptor protein. Estrogen receptors also modulate gene expression in the developing fetus and in adolescents during puberty.



Computational analysis shows the probability that three heterocyclic amines and one metabolite will compete with estradiol at the estrogen-receptor binding cavity. PhIP = 2-amino-1-methyl-6-phenylimidazol[4,5-*b*]pyridine; MeIQx = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; and IFP = 2-amino-1,6-dimethylfuro[3,2-*e*]imidazo[4,5-b]pyridine. N^2-hydroxy-PhIP is a PhIP metabolite.

on the Laboratory's Multiprogrammatic Capability Resource supercomputer, which can process 11 trillion operations per second. With high-resolution simulations, researchers can observe, in hours or days, the numerous positions the heterocyclic amines may assume relative to the estrogen receptor. By contrast, experiments would take months or even years to produce the same data.

For the modeling studies, the team obtained a structure for the estrogen receptor from the Protein Data Bank, an international repository for protein structures. Team members set up a control model by calculating the position coordinates of estradiol in the estrogen receptor's ligand-binding domain. They then used an algorithm to specify the number of docking, or binding, steps they wanted to observe and compared positions for PhIP, N^2-hydroxy-PhIP, IFP, and MeIQx with those for the control model.

The team's calculations showed that PhIP attaches directly to the estrogen receptor's ligand-binding domain. However, the probability of it doing so is less than that for estradiol. In addition, N^2-hydroxy-PhIP is oriented in the same docking position to the estrogen receptor as PhIP, but N^2-hydroxy-PhIP does not compete with estradiol for binding. Computational biologist Brian Bennion explains, "To compete with estradiol in the estrogen-receptor binding cavity, a heterocyclic amine or its metabolite must have the same orientation and arrangement of atoms. The hydroxy atoms in N^2-hydroxy-PhIP are not arranged in a way that would make it as easy as it is for PhIP to bind at the estrogen-receptor site. The other heterocyclic amines we examined, MeIQx and IFP, have three fused heterocyclic rings in their molecular structure, which limits interactions even more."

Tuning into a Molecule's Frequency

Experiments with NMR spectroscopy—one of the experimental methods used to determine protein structure for the Protein Data Bank—confirmed the simulation results that PhIP binds to the estrogen-receptor protein. With NMR, a molecule's nuclei will resonate to a unique radio frequency, providing physical, chemical, electronic, and structural information about the molecule. Livermore researchers have developed methods that use NMR in competition assays to study whether molecules compete for the same binding location on a protein.

Determining whether PhIP competes with estradiol for the estrogen receptor's ligand-binding domain was particularly challenging. "Estrogen receptors are sensitive proteins," says physical chemist Monique Cosman, who leads the Biomolecular NMR Group at Livermore. "For example, if we shake them or try to concentrate them, they will aggregate and form a precipitate, making it impossible to collect data." Another challenge is that some proteins are only active in a high-salt environment, but PhIP and estradiol are only soluble in a 100-percent organic solvent, such as dimethyl sulfoxide (DMSO). The two environments are compatible only at less than 5-percent DMSO.

To ensure that precipitate did not form, the team used low concentrations of estrogen receptor, PhIP, and estradiol and extended the run times for the NMR experiments. "A typical NMR experiment uses a sample concentration that is 100 to 1,000 times greater than we used," says Cosman. "To adjust for the low concentrations, we ran the experiments for four days to increase the number of scans collected. Then we averaged them over time to increase the signal-to-noise ratio. With this approach, we detected, for the first time, that PhIP and estradiol do in fact compete for binding in the same site on the estrogen receptor."

When examined with NMR, small molecules such as PhIP exhibit a weak, positive signal called the nuclear Overhauser effect (NOE). The much larger estrogen-receptor protein exhibits a strong, negative NOE signal. If a small molecule binds to a protein, the characteristics of the NOE for the protein will be transferred to the small molecule.

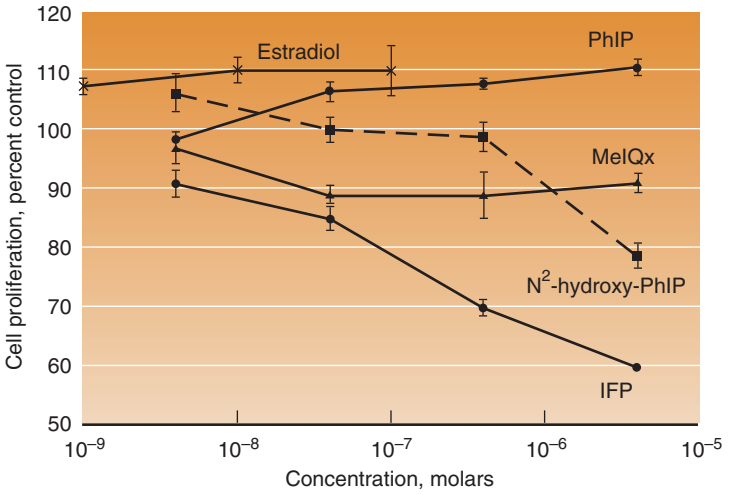
Adding PhIP to the sample solution changed the NOE signal for PhIP from positive to negative, indicating that PhIP was bound to the estrogen-receptor protein. When estradiol was added, the signals reverted to weakly positive. This change indicated that estradiol competitively displaced PhIP and that both ligands were binding to the same site on the protein. The NMR data thus confirmed the computational results that PhIP binds to the same site on the estrogen-receptor protein but not as strongly as estradiol.

Increased Growth of Cancer Cells

The Livermore team also examined whether heterocyclic amines influence cell proliferation. Using in vitro assays, researchers compared cells treated with a heterocyclic amine to untreated cells. This study indicated that treatment with only PhIP increases cell proliferation in human breast-cancer cells up to 40 percent and almost doubles estrogen-receptor activity compared with the activity in untreated cells. Results from the cell assays are consistent with those from the computer simulations, which predicted that the probability of PhIP binding to the estrogen receptor is almost as high as that of estrogen. In contrast, the cellular assays demonstrated that N^2-hydroxy-PhIP, MeIQx, and IFP inhibit estrogen-receptor activation, also consistent with the team's simulations.

One explanation of these results is that the binding mechanism for MeIQx and IFP differs from that for PhIP and N^2-hydroxy-PhIP. MeIQx and IFP may even bind in other regions of the estrogen-receptor protein that somehow prevent activation.

Kulp notes that the concentrations of PhIP examined by her team are orders of magnitude higher than what a single cell might be exposed to after a meal of cooked meat. However, prolonged exposure to PhIP over a lifetime may add to the total estrogenic burden on the body. In addition, the biological consequence of exposure to heterocyclic amines may differ when they are combined with other foods. Previous Livermore studies showed that the cooking method also affects the formation of the different heterocyclic amines. (See *S&TR*, July 1995, pp. 6–25; September 1995, pp. 6–23; April 2001, pp. 4–11.)



Assays of breast-cancer cells show that adding PhIP increases cell proliferation, as does the estrogen hormone estradiol, which regulates gene growth. In contrast, adding MeIQx, IFP, and N^2-hydroxy-PhIP do not increase proliferation.

The Livermore researchers are evaluating the possible benefits of soy and green tea to determine if exposure to such foods will inhibit the activation. They also want to expand their NMR research to examine other heterocyclic amines and hope to conduct additional studies on PhIP. "We have good evidence now that PhIP can bind to the same site as estradiol," says Cosman. "Next, we're planning studies to directly map its orientation at the binding site."

By demonstrating through experiments and simulations that PhIP can activate estrogen receptors and stimulate breast-cancer cell proliferation, the Livermore team is helping to determine how dietary constituents may affect the growth of hormone-sensitive cancers. More importantly, better understanding of these mechanisms may lead researchers to develop potent therapeutics for treating breast cancer.

—Gabriele Rennie

Key Words: 2-amino-1,6-dimethylfuro[3,2-*e*]imidazo[4,5-*b*]pyridine (IFP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazol[4,5-*b*]pyridine (PhIP), estradiol, estrogen-receptor protein, food mutagen, heterocyclic amine.

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